SWD in: (i) a rat genetic model of absence seizures (intrathalamic administration) [Z. Liu, M. Vergnes, A. Depaulis, C. Marescaux, *Neuroscience* 48, 87 (1992)] and (ii) two pharmacologic models of absence seizures (i.p. administration) [O. C. Snead, *Eur. J. Pharmacol.* 213, 343 (1992)].
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28. B63HF1 is the F_1 from C57Bl/6JEi females crossed with C3H/HESnJ males. We refer to the B63HF1 strain, with which *lh/lh* is congenic, as wild or +/+. We maintain *lh/lh* and +/+ mouse colonies at the Duke University Vivarium, using stocks from Jackson Laboratory (Bar Harbor, ME).
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Nitric Oxide: A Physiologic Mediator of Penile Erection

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Nitric oxide (NO) is a cytotoxic agent of macrophages, a messenger molecule of neurons, and a vasodilator produced by endothelial cells. NO synthase, the synthetic enzyme for NO, was localized to rat penile neurons innervating the corpora cavernosa and to neuronal plexuses in the adventitial layer of penile arteries. Small doses of NO synthase inhibitors abolished electrophysiologically induced penile erections. These results establish NO as a physiologic mediator of erectile function.

Nitric oxide mediates bactericidal and tumoricidal actions of macrophages (*1*) and blood vessel relaxation of endothelial cells (*2*). NO may also be a major neuronal messenger (*3*). Immunohistochemical studies localize NO synthase (NOS) to neurons in the brain as well as to discrete populations of autonomic nerves in the periphery (*4*), where NO fulfills most characteristics of a neurotransmitter. For instance, NOS is highly localized to cell bodies and fibers of the myenteric plexus of the gastrointestinal pathway (*4*). The nonadrenergic, noncholinergic relaxation evoked by physiologic stimulation of myenteric plexus neurons is potently and selectively blocked by NOS inhibitors (*5*).

Penile erection is thought to involve parasympathetic, neuronally mediated relaxation of the blood vessels as well as of the trabecular meshwork of smooth muscle that comprises the corpora cavernosa (*6*). The neuronal chemical mediator of erection has not been established. Vasoactive intestinal polypeptide (VIP) occurs in limited populations of nerve fibers in the penis (*7*), but direct administration of VIP does not fully mimic physiologic erection (*8*). In isolated smooth muscle from the corpus cavernosa of several species, relaxation evoked by electrical field stimulation could be blocked by NOS inhibitors, as reported in some studies (*9*) but not in others (*10*). Blockage of relaxation by NOS inhibitors can establish NO as a mediator of cavernosal muscle relaxation but does not permit conclusions as to whether it is a neuronal, transmitter-like messenger and a physiologic mediator of erection.

Several portions of the genitourinary tract of rat displayed substantial NOS activity, monitored by the conversion of [^{3}H]arginine to [^{3}H]citrulline (Table 1). High concentrations in the pelvic plexus, referred to in the rat as the major pelvic ganglion, suggest a neuronal role for NOS. Amounts of NOS in the membranous urethra exceeded amounts in the pelvic plexus and were three to four times larger than those in the penis and the bladder neck and considerably larger than those in the prostate. This regional distribution of NOS activity was confirmed by protein immunoblot (*11*).

We conducted immunohistochemical staining of rat penile tissue (Fig. 1) with an antiserum that is highly selective for NOS and stains NOS specifically in a variety of rat peripheral tissues and in brain tissue (*4*). All immunohistochemical staining of NOS in penile tissues was blocked by preabsorption with recombinant NOS protein (*11*).

The antibody to NOS stained the pelvic plexus and its axonal processes that form the cavernous nerve (Fig. 1A), located immediately adjacent to the deep cavernosal artery, the major arterial source of the corpus cavernosum. In the proximal penis, the nerve plexus in the adventitia of the deep cavernosal arteries stained prominently as did neuronal processes in the sinusoids and the periphery of the corpora cavernosa (Fig. 1B). This staining circumscribed the corpora cavernosa directly below their fibrous capsules, the tunica albuginea. Neuronal staining of the deep cavernosal arteries continued as the arteries subdivided into the intracorporal network of helicine arteries (Fig. 1D). In the most distal portion of the corpora, staining diminished as the helicine arteries were replaced with cavernous spaces (Fig. 1E). The distal part of the penis superficial to the corpora cavernosa contained dorsal penile nerve fibers that stained for NOS (Fig. 1, E and F). Dorsal penile and cavernosal arteries stained for NOS both in the adventitial and endothelial layers, although endothelial staining was faint in the cavernosal vessels. NOS staining in the urethra was associated with neuronal fibers coursing through the smooth muscle or the submucosal vasculature or both (Fig. 1E) and fits with the substantial urethral NOS catalytic activity (Table 1).

The neural specificity of NOS staining was established by bilateral cavernous nerve transection, after which we no longer observed penile neurons stained for NOS (Fig. 1C), although endothelial staining persisted (*11*). To ensure that this distribution was not species-specific, we conducted immunohistochemical localizations of NOS from the penes of dogs and demonstrated essentially identical localizations to nerve plexuses in the adventitial layers of penile arteries and the dorsal nerves of the dog penes.

The localization of NOS to neuronal fibers innervating blood vessels and the corpora cavernosa of the penis suggested a possible role for NO as a neuronal mediator of erection. We examined this possibility in a rat model of penile erection in which we electrically stimulated the cavernous nerves of intact rats by using optimal parameters that evoked physiologic erection (*12*). L-Nitroarginine, a potent and selective inhibitor of NOS, markedly diminished penile erections (Table 2). As little as 1 mg per kilogram of body weight (mg/kg) administered intravenously (i.v.) significantly reduced erection, and 2.5 mg/kg produced more than a 50% reduction. At 5 mg/kg, nitroarginine almost completely inhibited erection. Intravenous bolus injections of

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L-arginine (25 mg/kg) partially reversed the L-nitroarginine (2.5 mg/kg) inhibition of penile erection. The physiologic L-isomer of *N*-methylarginine, another selective inhibitor of NOS that is less potent than L-nitroarginine, significantly inhibited penile erection at 10 mg/kg with a larger effect of 40 mg/kg. By contrast the D-isomer, which does not inhibit NOS, also failed to block erection even at 40 mg/kg. Large doses of atropine (1 mg/kg i.v.) did not inhibit electrically stimulated erections.

These results fit with recent experiments that show inhibitions of erection by cavernosal nerve stimulation in rabbits after injections of L-nitroarginine directly into the corpus cavernosum (*13*). The dose required for maximal effect, about 2 mg, is effectively several hundred times greater than the parenteral doses we administered to rats. Also, L-arginine failed to reverse effects of nitroarginine in rabbits (*13*).

The stereospecificity for inhibition of penile erection displayed by *N*-methylarginine as well as the very substantial potency of nitroarginine indicates that the blockage of penile erection comes from the inhibition of NOS activity. This conclusion is supported by observations that relaxation of isolated corpus cavernosum muscle strips in vitro after electrical field stimulation is blocked by NOS inhibitors (*9*), although others report a failure of the inhibitor *N*-methylarginine to prevent such relaxation (*10*). The selective localization of NOS in penile neurons that subserve erection, as well as the ability of NOS inhibitors to block physiologic erection selectively, potently, and completely, imply that NO is the major if not sole neuronal mediator of erection. As in the myenteric plexus of the gastrointestinal system, NO in the penis appears to fulfill the principal criteria of a neurotransmitter. For instance, it is localized to the neurons that innervate the smooth muscle of the penis. Furthermore, direct application of NO or its precursors relaxes the muscle in a manner similar to the relaxation produced by nerve stimulation (*9*), and the effects of neuronal stimulation are blocked by inhibitors of the formation of NO.

Table 1. NOS activity in the urogenital system. We measured the activity of NOS by monitoring the conversion of [^{3}H]arginine to [^{3}H]citrulline as described (*21*) on specimens obtained by anatomical dissection of adult male Sprague-Dawley rats. Tissue was homogenized in 10 volumes (w/v) 50 mM tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, and centrifuged at 10,000*g* for 1 min at 4°C. Enzyme assays contained 25 μl of tissue supernatant and 50 μl of 100 nM [^{3}H]arginine (53 Ci/mmol; 1 Ci = 37 gigabecquerels), 10 mM nicotinamide adenine dinucleotide phosphate (reduced form), and 10 mM $CaCl_2$. After a 15-min incubation at room temperature, the assays were terminated with 3 ml of 20 mM Hepes (pH 5.5) with 2 mM EDTA and applied to 0.5-ml columns of Dowex AG50WX8 (Na^+ form). [^{3}H]Citrulline was quantified by liquid scintillation spectroscopy of the 3-ml flow-through. The data are expressed as mean values ± SEM for five experiments and normalized to cerebellar NOS activity assayed in parallel. According to the Duncan multiple range test, the amounts of NOS catalytic activity in the pelvic plexus and urethra are different from the amounts in the prostate, bladder neck, and penis.

Structure	[^{3}H]Citrulline formation [cpm min^{-1} $(mg\ protein)^{-1}$ ± SEM]
Pelvic plexus	408 ± 36
Membranous urethra	857 ± 105
Penis	212 ± 44
Bladder neck	214 ± 31
Prostate	36 ± 15

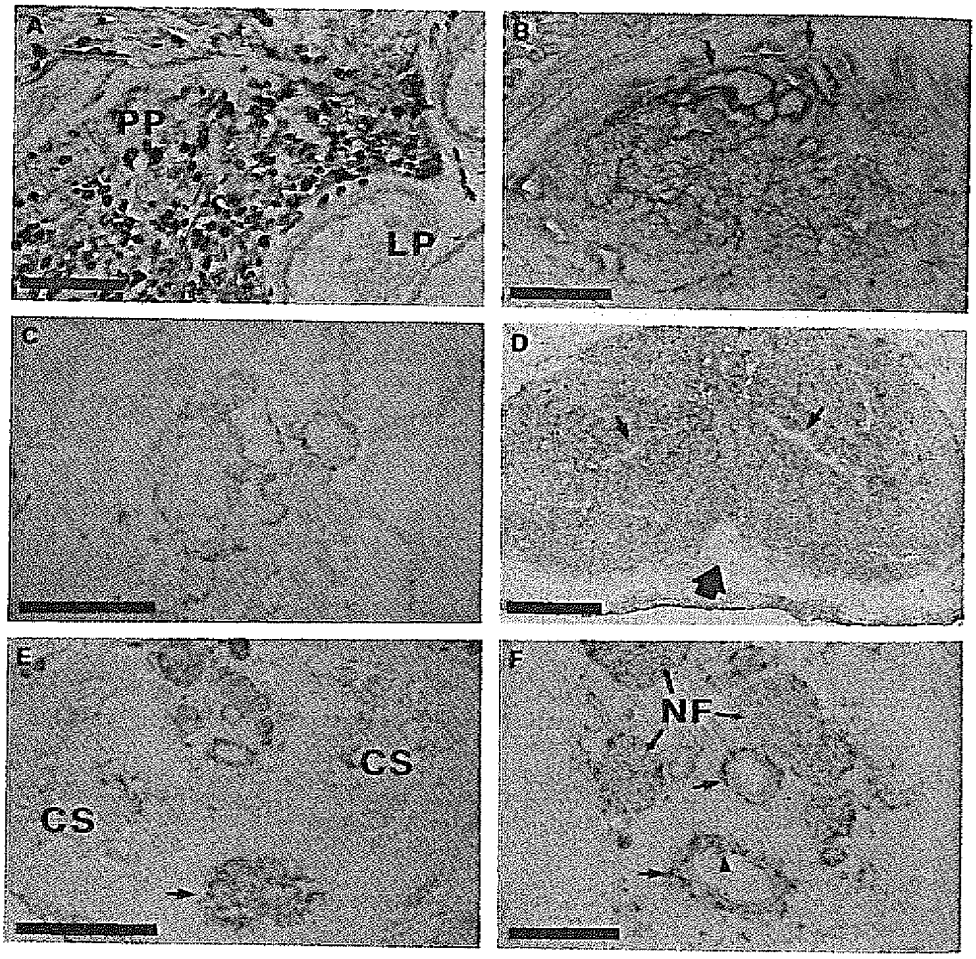

Fig. 1. Immunohistochemical localization of NOS in the rat penis. Immunohistochemistry was performed as described (*4*) on slide-mounted pelvic tissue sections from adult male Sprague-Dawley rats. The primary antibody was an affinity-purified NOS antiserum (1:50 dilution), which was subsequently bound with the use of an avidin-biotin-peroxidase system (Vector Laboratories) with diaminobenzidine as a chromogen. (**A**) The pelvic plexus (PP) containing neural cell bodies adjacent to glands of the lateral prostate (LP). (**B**) Oblique section through the crus of the corpus cavernosum that depicts staining primarily localized to nerves in the adventitia of the deep cavernosal artery and its major tributaries (arrows) and to nerves extending into erectile tissue. (**C**) Duplicate section as (B) that was obtained in an animal 1 week after bilateral transection of the cavernous nerves. Faint staining can be observed in the adventitia of the major arterial divisions. (**D**) Coronal section through the pelvis at the level of the proximal penis that shows the corpora cavernosa merging in midline. The deep cavernosal arteries have tapered (arrows), whereas arterial subdivisions, the helicine arteries, have extensively arborized. The capsule containing the erectile tissue, the tunica albuginea, does not stain (thick arrow). (**E**) Cross section through the visible penis distally showing prominent nerve fiber staining of the dorsal penis and staining of the urethra (arrow). Bilateral cavernous spaces (CS) within the corporal bodies are shown. (**F**) A magnified view of the dorsal penis from (E) showing discrete nerve fibers (NF). Staining is also localized to the adventitia (arrows) and endothelium (arrowhead) of dorsal arteries. [Scale bars in (A) to (C), and (F) = 300 μm; bars in (D) and (E) = 1 mm].

Table 2. Effects of NOS inhibition on penile erection in intact rats. Penile erection was induced electrically with a Grass S48 square wave stimulator in anesthetized (pentobarbital, 50 mg/kg, administered intraperitoneally) male Sprague-Dawley rats with optimal stimulation parameters (*12*). Bipolar silver wire electrodes were attached unilaterally to the cavernous nerve that arises from the ipsilateral pelvic plexus situated dorsolateral to the prostate. Intracavernous pressures were measured (Gould Polygraph, Cleveland, Ohio) with a 25-gauge needle inserted unilaterally at the base of the penis and connected to an Isotec pressure transducer. Neurostimulation was performed until a 10-s maximal pressure recording was achieved, but no stimulation lasted longer than 90 s. At least 10 min elapsed between repeated stimulations. Arginine derivations were administered into the jugular vein. Data represent mean values ± SEM as a percentage of the baseline pressure (range 35 to 50 mmHg) recorded 15 min after agents were administered.

Agent	Dose (mg/kg)	% Intracavernous pressure (± SEM)	*n*
L-Nitroarginine methyl ester	1.0	75 ± 7	3
	2.5	47 ± 5	10
	5.0	16 ± 1	4
	10.0	10 ± 10.3	3
	40.0	0	5
N-Methyl-L-arginine	10.0	63 ± 3	11
	20.0	17 ± 2	2
	40.0	15 ± 5	4
N-Methyl-D-arginine	40.0	128 ± 5	2

Acetycholine is the classical neurotransmitter for the parasympathetic innervation of penile nerves responsible for erection. However, penile erection does not appear to require either cholinergic or adrenergic mechanisms (*14*). VIP was advanced as a candidate transmitter for the mediation of erection on the basis of its immunohistochemical localization in penile neurons (*7*). However, the density of VIP-containing penile neurons in several species (*8*) is substantially less than that of the NOS-containing neurons that we have observed in rats and dogs. Injections of VIP into the dog and human penis produce some erection, but responses are relatively modest and may be elicited primarily by increased venous outflow resistance rather than by the dilation of penile arteries or by the relaxation of corpora cavernosa muscle (*8*). Lesion studies demonstrate that NOS-containing fibers in the adventitia of cerebral arteries come from parasympathetic cell bodies in the sphenopalatine ganglia, many of which also contain VIP (*15*). In the myenteric plexus, NOS-containing neurons also contain VIP. Whereas NOS inhibitors can almost completely block neuronally mediated gastric relaxation, antibodies to VIP can produce up to a 30% blockage of this relaxation with NOS inhibitors blocking the remaining relaxation (*16*). Thus, in various portions of the parasympathetic nervous system, VIP and NO might function as cotransmitters.

The immunohistochemical visualization of NOS in penile neurons clarifies functional penile innervation as described in humans by Walsh and Donker (*17*) and as described by others in rats (*18*). NOS-containing cavernous nerve processes penetrate the corpora cavernosa, appear to envelop the centrally situated cavernosal arteries, and also extend into the corporal bodies radially and circumferentially. This implies a direct neural modulation of the vasoactivity of penile arteries, intracorporal sinusoids, and the entire tubular-shaped corpora cavernosa. Besides well-characterized neuroregulation of arterial dilation in the penis, our findings suggest that penile erection also involves an active process of neurally regulated sinusoidal and corporal expansion rather than passive engorgement of cavernous spaces with blood supplied by the penile arteries. The immunohistochemical localization of NOS in the urethra parallels its high NOS catalytic activity, which suggests that NO may be involved in the urethral functions that regulate urinary continence or micturition.

The involvement of NO in erection has clinical implications. Priapism, a condition of painful, prolonged erections unassociated with sexual arousal or desire, occurs in several clinical situations, including as many as 40% of patients with sickle cell anemia (*19*). NOS inhibitors such as nitroarginine might have therapeutic utility in priapism. Furthermore, some varieties of impotence may result from a dysfunction in the NOS-containing neuronal system. Our delineation of NOS neuronal localizations within the corpora cavernosa may facilitate surgical approaches to correct penile abnormalities and injuries, such as hypospadias and Peyronie's disease, respectively congenital and acquired conditions conspicuous for disabling penile curvature. Impotence associated with their operative management (*20*) might be avoided with surgical techniques designed to preserve NOS innervation.

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